IN THE CLAIMS:

A listing of the claims, in accordance with the revision of 37 CFR §1.121, is provided. The listing of claims replaces all prior such listings of claims. Claims 1, 3, 61, 63 and 71 are amended herein. Claims 21, 62, 72, 79-84 and 86 are cancelled herein without prejudice or disclaimer.

Listing of claims:

1. (Currently amended) A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating tumor cells in a sample of a body fluid by covering a cell separation medium with a density in the range of from 1.060-1.067 g/ml with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid;

(b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; [[and]]

(c) quantitatively determining the amount of amplified nucleic acid~~, thereby quantifying tumor cells in a body fluid.~~; and

(d) correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid.

2. (Previously presented) The method of Claim 1, further comprising prior to amplification, preparing cDNA from the mRNA contained in the sample.

3. (Currently amended) The method of Claim 2, wherein, prior to preparing cDNA, [[the]] the sample is treated with a DNAase.

4. (Previously presented) The method of Claim 1, wherein the sample gel is purified.

5. (Previously presented) The method of Claim 1, wherein for quantitative determination of the telomerase-coding nucleic acid, the amplification products are labeled during amplification and the amplification kinetics are measured continuously, including during the amplification process.

6. (Previously presented) The method of Claim 5, wherein a probe that is specific for the amplification products, and that emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification.

7. (Previously presented) The method of Claim 1, wherein for quantitative determination of the telomerase-encoding nucleic acid, at least one standard nucleic acid molecule is coamplified and added in different concentrations to the sample.

8. (Previously presented) The method of Claim 1, wherein the amplification product is quantified either directly or via a label.

9. (Previously presented) The method of claim 1, wherein the amplification product is detected via hybridization with a labeled oligonucleotide.

10. (Previously presented) The method of Claim 7, wherein quantification of the telomerase-encoding nucleic acid is effected by comparing the amount of coamplified nucleic acid or nucleic acids with the amount of telomerase-encoding nucleic acid.

11. (Previously presented) The method of claim 1, wherein the sample is peripheral blood.

12. (Previously presented) The method of Claim 1, wherein as a negative control water is employed in place of the body fluid.

13. (Previously presented) The method of Claim 1, wherein one or both of the following oligonucleotide primers are used for the amplification:
5′ CTACCGGAAG AGTGTCTGGA GCAAGTTGGA AAGC 3′ SEQ ID No. 1, designated TRT1; and
5′ GGCATACCGA CGCACGCAGT ACGTGTTCTG 3′ SEQ ID No.2, designated TRT2,

wherein each of hTRT1 and hTRT2 optionally further comprises a promoter sequence for an RNA polymerase.

14. (Previously presented) The method of Claim 1, wherein amplification is effected with a DNA polymerase or an RNA polymerase

15. (Previously presented) The method of claim 14, wherein, if amplification is effected with a DNA polymerase, the amplification reaction is a polymerase chain reaction (PCR)and, if amplification is effected with an RNA polymerase, the reaction is an isothermal nucleic acid sequence-based amplification (NASBA) reaction.

16. (Previously presented) The method of claim 1, wherein the sample is blood that is depleted in stem cells and/or activated immune cells.

17. (Previously presented) The method of Claim 1, wherein the sample is blood, and the tumor cells from the blood sample are concentrated.

18. (Previously presented) The method of Claim 1, wherein the cells contained in the sample are cultivated under conditions that are unfavorable for telomerase-positive nontumor cells but favorable for the tumor cells present.

19. (Previously presented) The method of Claim 18, wherein the duration of the cultivation is such that nontumor cells die and tumor cells survive.

20. (Canceled)

21. (Canceled)

22. (Previously presented) The method of Claim 1, wherein the centrifugation is carried out at about 1000 x g for about 30 minutes.

23. (Previously presented) The method of Claim 1, wherein the cell separation medium used is Percoll or Ficoll.

24. (Previously presented) The method of Claim 1, wherein the body fluid is blood and prior to applying the body fluid sample to the cell separation medium, the body fluid is mixed with one or more substances that prevent aggregation of platelets to tumor cells, and/or prior to applying the body fluid sample to the cell separation medium, the body fluid is freed of substances that promote aggregation of platelets to tumor cells.

25. (Canceled)

26. (Previously presented) The method of Claim 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent.

27. (Previously presented) The method of Claim 11, wherein the peripheral blood is venous or arterial blood.

28. (Previously presented) The method of Claim 1, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

29. (Previously presented) The method of Claim 1, wherein after centrifugation and before collecting the tumor-cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers.

30. (Previously presented) The method of Claim 1, wherein the centrifugation is carried out in a vessel that is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment and the body fluid is introduced into the upper compartment.

31. (Previously presented) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a thickness of 1-10 mm.

32. (Previously presented) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a pore size of 20-100 μm.

33. (Previously presented) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve is fabricated from a hydrophobic material or coated with a hydrophobic material.

34. (Previously presented) The method of Claim 1, wherein a dye is added to color the cell separation medium, whereby the color of the cell separation medium is distinguishable from that of the supernatant body fluid.

35. (Previously presented) The method of Claim 1, wherein: the sample is blood;

the method is performed on venous blood sample and on an arterial blood sample; and the results from each are compared with one another.

36. (Previously presented) The method of Claim 1, wherein: the sample is blood;

the method is performed on a blood sample from the finger pad and, on a venous or arterial blood sample; and

the results from each are compared with one another.

37. (Previously presented) The method of Claim 1, wherein the tumor cells are derived from metastases of malignant tumors.

38. (Previously presented) The method of Claim 1, wherein the tumor cells are selected from cells of metastasizing tumors and/or neoplasms, wherein the cells are derived from tumors and cells selected from the group consisting of a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and lymphoma cells.

Claims 39-51 (Canceled)

52. (Original) The method of Claim 4, wherein purification is effected by ion exchange chromatography.

53. (Original) The method of Claim 52, wherein ion exchange resin is a silica gel.

54. (Original) The method of Claim 7, wherein three standard nucleic acids are coamplified and are added in different concentrations to the sample.

55. (Original) The method of Claim 8, wherein:

the amplification product is quantified via a label; and

the label is selected from a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

56. (Original) The method of Claim 9, wherein the label is a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

57. (Original) The method of Claim 11, wherein, as a positive control in the sample, a nucleic acid that occurs in peripheral blood is specifically amplified and detected.

58. (Original) The method of Claim 57, wherein the nucleic acid is mRNA that encodes a protein selected from among β-globin, glyceraldehyde-phosphate dehydrogenase, β-actin or a T-cell receptor.

59. (Original) The method of Claim 3, wherein as a negative control no reverse transcription reaction is carried out before the amplification reaction with the sample to be investigated and/or water is employed in place of the body fluid.

60. (Original) The method of Claim 16, wherein depletion is effected by immunoabsorption.

61. (Currently amended) The method of Claim [[78]] 17, wherein concentration is effected by immunoabsorption.

62. (Cancelled)

63. (Currently amended) The method of Claim [[62]] 1, wherein the density is about 1.065 g/ml.

64. (Previously presented) The method of Claim 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1.

65. (Original) The method of Claim 31, wherein at least one of the porous barrier, the filter or the sieve has a thickness of about 5 mm.

66. (Original) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a pore size of 20-30 μm.

67. (Original) The method of Claim 1, wherein the tumor cells are derived from micrometastases of malignant tumors.

68. (Canceled)

69. (Previously presented) The method of Claim 1, wherein the tumor cells are separated from telomerase-positive non tumor cells.

70. (Previously presented) The method of Claim 1, further comprising, before step (i), adjusting the density of the cell separation medium and thereby separating tumor cells from telomerase-positive non tumor cells.

71. (Currently amended) A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating and separating the tumor cells from telomerase-positive non-tumor cells in a sample of a body fluid;

(b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and

(c) quantitatively determining the amount of amplified nucleic acid~~, thereby quantifying tumor cells in a body fluid.~~; and

(d) correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid.

72. (Cancelled)

73. (Original) The method of Claim 71, further comprising prior to amplification, preparing cDNA from the mRNA contained in the sample.

74. (Original) The method of Claim 71, wherein one or both of the following oligonucleotide primers are used for the amplification:
5' CTACCGGAAG AGTGTCTGGA GCAAGTTGGA AAGC 3' SEQ ID No. 1, designated TRT1; and
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' SEQ ID No.2, designated TRT2,

wherein each of hTRT1 and hTRT2 optionally further comprises a promoter sequence for an RNA polymerase.

75. (Original) The method of Claim 71, wherein step (a) comprises:

(i) covering a cell separation medium with a layer of the body fluid;

(ii) centrifuging the cell separation medium covered with the body fluid; and (iii) collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid.

76. (Original) The method of Claim 71, wherein the tumor cells are selected from cells of metastasizing tumors and/or neoplasms, wherein the cells are derived from tumors and cells selected from the group consisting of a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and lymphoma cells.

77. (Original) The method of Claim 75, wherein the cell separation medium has a density in the range of from 1.055 to < 1.070 g/ml.

78. (Original) The method of Claim 71, wherein the sample is blood, and the tumor cells from the blood sample are concentrated.

Claims 79-84 (Canceled)

85. (Original) The method of Claim 71, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

86. (Canceled)

REMARKS

Any fees that may be due in connection with filing this paper or with this application may be charged to Deposit Account No. 50-1213. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-19, 22-24, 26-38, 52-61, 63-67, 69-71, 73-78 and 85 are pending. Claims 21, 62, 72, 79-84 and 86, are cancelled without prejudice or disclaimer. Applicant reserves the right to file divisional applications to any cancelled subject matter.

Claims 1, 3, 61, 63 and 71 are amended. Claim 3 is amended to correct a minor typographical error, and Claim 61 is amended to correct claim dependency. Basis for Claim 61 is in the specification, *e.g.*, at page 15, line 35 to page 16, line 2. Claim 1 is amended to incorporate the limitations of Claim 62 and Claim 63, which was formerly dependent on Claim 62, is accordingly amended to depend from Claim 1.

Claims 1 and 71 are amended to include a positive step of quantification of tumor cells. As amended, Claims 1 and 71 recite a step of "correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid." Basis for this amendment can be found in the specification, for example, at page 14, lines 24-26 and at page 26, lines 3-11, which provides that quantitative determination of the mRNA that codes for the catalytic subunit of telomerase can be used to obtain the number of tumor cells circulating in a body fluid, which in turn is indicative of metastasis. As addressed in an Amendment mailed February 25, 2002, responsive to an Office Action mailed October 24, 2001, by a previous Examiner, it was routine and well-known to those of skill in the art at the time of filing of the instant application (and well before) that when a particular gene or nucleic acid in a cell is amplified and quantified, it is possible to deduce the number of cells therefrom.

Further, as also discussed earlier in the Amendment mailed February 25, 2002, the Figures and Examples in the specification provide detailed descriptions and demonstrations of how a correlation can established between

the amount of amplified catalytic subunit of telomerase and the quantity of tumor cells. For example, Figure 5 of the specification shows a correlation between the amount of amplified product of the mRNA for the catalytic subunit of telomerase and the number of LNcap prostate carcinoma cells. Figure 5 demonstrates that the mRNA coding for the catalytic subunit of telomerase can be detected in as few as 10 cells. Figure 8 and the Examples section of the specification at page 32 demonstrates that amplification of the mRNA for the catalytic subunit of telomerase detects as few as 2 melanoma cells per ml of blood, when compared to blood that does not contain melanoma cells. Thus, there is adequate basis in the specification for a step of correlating the amount of amplified mRNA for the catalytic subunit of telomerase with the quantity of tumor cells in a body fluid. Furthermore, such correlation is routine, since one of skill in the art could determine the amount of mRNA by comparison with a standard.

No amendments have been made to obviate prior art and no new matter is added. The claims are amended to render it clear that tumor cells are separated from telomerase positive non-tumor cells, and the amount of mRNA encoding the catalytic subunit of telomerase is assessed and correlated with the number of tumor cells. As discussed in detail below with respect to each rejection, none of the cited references, singly or in any combination thereof, teaches or suggests a method in which there is a separation of tumor cells from telomerase positive non-tumor cells and a correlation of mRNA encoding the catalytic subunit of telomerase with the number of tumor cells.

STATEMENT OF THE SUBSTANCE OF THE INTERVIEW OF AUGUST 25, 2003, WITH THE EXAMINER

Applicant thanks the Examiner for the courtesy extended in granting an interview to discuss specific issues raised in the Final Office Action of July 8, 2003. Pursuant to the discussion, Applicant further thanks the Examiner for agreeing to consider the instant Amendment after Final that incorporates the Examiner's suggestions provided during the aforementioned interview.

In the interview, Applicant discussed the Examiner's rejection of the pending claims under 35 U.S.C. §103(a) over Cech *et al.* alone or in combination with various secondary references, in particular Van Vlasselaer *et al.*. Responsive to the Examiner's allegation in the Final Office Action that Cech *et al.* teaches each of the positive process steps of the instantly claimed methods because the step of "quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid," is not a positive process step of quantification of tumor cells. It was agreed that amendment of the claims to recite a positive step of correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid should obviate this rejection.

Applicant further pointed out that Cech *et al.*, either alone or in combination with various cited references, including Van Vlasselaer *et al.*, does not teach or suggest any method that includes a step of separating tumor cells from telomerase-positive non-tumor cells. Applicant proposed filing an amended set of claims (Claim 1 and dependents) that included a separation medium density range of "1.060-1.067 g/ml," which range is necessary for separation of tumor cells from telomerase-positive non-tumor cells.

In considering Applicant's aforementioned proposal, the Examiner also reviewed pending Claims 70 and 71, which recite a step of separating tumor cells from telomerase-positive non-tumor cells. The Examiner indicated independent Claim 71 and dependents as being suitable for consideration in an Amendment after Final. The Examiner further encouraged Applicant to clearly point out in the response the deficiencies of each of the cited references and why it would not be obvious to separate tumor cells from telomerase-positive non-tumor cells. The Examiner advised Applicant against adding any new limitations that would require new search or consideration.

It is respectfully submitted that, as discussed below, the instant Amendment after Final is compliant with the Examiner's requirements as set forth during the interview. It is further submitted that the instant Amendment

after Final is fully responsive to the Final Office Action of July 8, 2003, and either places the application into condition for allowance, or, alternatively, reduces the number of issues for appeal.

CLAIMS 73-86

The Final Office Action states that Claims 1-19, 21-24, 26-38, 52-67 and 69-72 are pending. No mention is made of Claims 73-86, also pending as of the last Amendment mailed May 6, 2003. In the Amendment mailed May 6, 2003, responsive to the previous Office Action of November 6, 2002, Applicant added claims 71-86. In the instant Final Office Action, while the Examiner has acknowledged added independent Claims 71 and 72, dependent Claims 73-86 have not been listed as pending. Applicant requests clarification as to whether Claims 73-86, listed in the Amendment mailed May 6, 2003, have been made of record in the file history of the above-captioned application. In the event that the aforementioned claims were not so entered, Applicant requests that they be made of record as set forth in the Amendment mailed May 6, 2003. It is noted, however, that regardless of the status of Claims 73-86, Applicant's traversal of the rejection of Claims 1-19, 21-24, 26-38, 52-67 and 69-72 under 35 U.S.C. §103(a) (*see* below), is also applicable to Claims 73-78 and 85 that have not been so rejected. With regard to Claims 79-84 and 86, this rejection has been rendered moot by cancellation of these claims herein.

RESPONSE TO EXAMINER'S COMMENTS THROUGHOUT THE FINAL OFFICE ACTION THAT APPLICANT ATTACKED THE CITED REFERENCES INDIVIDUALLY IN ADDRESSING REJECTIONS UNDER 35 U.S.C. §103(a)

Prior to addressing the rejections under 35 U.S.C. §103(a), it is noted that in the instant Final Office Action, the Examiner alleges that in the Amendment and Response filed May 6, 2003, Applicant addressed some of the rejections (rejection of Claim 29 over Cech *et al.* in view of Selby *et al.*; rejection of Claims 5 and 6 over Cech *et al.* in view of Gelmini *et al.*; rejection of Claims 12 and 57-59 over Cech *et al.* in view of Melvin *et al.*; rejection of Claims 30-33, 65-66 over Cech *et al.* in view of Van Vlasselaer and further in

view of Oka *et al.*) by attacking the cited references individually, and that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references.

It is respectfully submitted that Applicant did not attack the references individually. Rather, Applicant discussed the teachings of each cited reference individually, then demonstrated that the combination of the cited references did not result in the instantly claimed subject matter. In the previous Amendment Applicant had organized arguments responsive to the rejection under 35 U.S.C. §103(a) as follows:

1) The pending claims.
2) Teachings of each of the cited references.
3) Arguments that the combined teachings of the cited references do not lead to the instantly claimed subject matter.

In the instant Amendment after Final, pursuant to discussions with the Examiner during the aforementioned interview of August 25, 2002, Applicant has provided arguments that the combined teachings of the cited references does not lead to the instantly claimed methods, all of which include steps of correlating the amount of amplified mRNA encoding the catalytic subunit of telomerase with the number of tumor cells in a body fluid and a step of separating tumor cells from telomerase-positive non-tumor cells or of reciting a particular cell separation medium density range that effects such separation.

THE REJECTION OF CLAIMS 1-19, 21-24, 26-38, 52-67 AND 69-72 UNDER 35 U.S.C. § 103(a)

A. REJECTION OF CLAIMS 1, 2, 4, 7-11, 14-19, 21-24, 26-28, 34-37, 38, 52-56, 60-64, 67 and 69-72 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER

Claims 1, 2, 4, 7-11, 14-19, 21-24, 26-28, 34-37, 38, 52-56, 60-64, 67 and 69-72 are rejected under 35 U.S.C. §103(a) over Cech *et al.* in view of Van Vlasselaer (U.S. Patent No. 5,648,223) because Cech *et al.* allegedly teaches a method of quantitating tumor cells in a body fluid, specifically, (1)

methods of diagnosing cancer in a patient by detecting a catalytic subunit of telomerase (hTRT) gene product in a biological sample (exemplified at col. 104, lines 65-68) obtained from the patient (col. 6, lines 20-40); **(2)** that detection of the hTRT gene, mRNA or protein level above a standard range is indicative of the presence of telomerase-positive cells such as tumor cells (col. 99, lines 5-20); **(3)** that cells or tissues may be fractionated, *e.g.*, by a cell sorter, prior to analysis (col. 105, lines 10-12; **(4)** primers useful for PCR amplification of hTRT (col. 107, lines 1-5); **(5)** analysis of the PCR amplified products (col. 108, lines 1-5); and **(6)** co-amplification reactions for normalization of the amount of hTRT in the sample to the cell number in the sample (col. 108, lines 45-65); and Van Vlasselaer allegedly teaches methods for enriching tumor cells by adjusting the density of cell separation media that are "routinely used in the art", such as Percoll and Ficoll, according to the density of the cell type. The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art to have modified the method of quantitation of tumor cells allegedly taught by Cech *et al.* with the tumor cell enrichment method using a cell separation medium allegedly taught by Van Vlasselaer, to arrive at the instantly claimed subject matter. Specifically, it is alleged that Van Vlasselaer teaches (1) Percoll and Ficoll (recited in instant claim 23) as cell separation media that are "routinely used in the art;" (2) providing a substance that prevents platelets from sticking to the tumor cells (recited in claim 24) and to remove the platelets as "routinely practised in the art" (3) that the density of the cell separation medium is adjusted to the density of the cell type, where the cell density is determined by "routine experimentation" (limitations of instant claims 21, 22, 24 and 62-64); and (4) that peripheral blood can be collected in anti-coagulant containing tubes (recited in claims 24-27).

Responsive to Applicant's arguments that neither of the cited references, singly or in combination, teaches or suggests the instant methods for quantification of tumor cells in a body fluid, nor (as recited in Claims 70 and 71) a step of separating tumor cells from telomerase-positive non-tumor cells, the

Examiner rebuts that Cech *et al.* teaches each of the "positive process steps" of the instantly claimed methods for the quantification of tumor cells because Cech *et al.* allegedly teaches quantifying the amount of amplified nucleic acid and the language "thereby" in the step of the instant methods that recites "quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid" is allegedly not a positive process step. It is further alleged that as to a step of separating tumor cells from telomerase positive non-tumor cells (Claims 70 and 71, *e.g.*), one of skill in the art would have had a reasonable expectation of success for separating various cells based upon density because Van Vlasselaer allegedly provides "specific methods for determining the specific density of a given tumor cell."

Reconsideration and withdrawal of this rejection is respectfully requested in view of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claims 21, 62 and 72, which have been cancelled.

Analysis

As discussed during the interview of August 25, 2003, the claims have been amended to recite a positive step of quantitation of tumor cells. As amended, Claims 1 and 71 and claims dependent thereon are directed to methods for the quantification of tumor cells in a body fluid in which the tumor cells are concentrated by centrifugation; mRNA coding for the catalytic subunit of telomerase is specifically amplified from the tumor cells; the amount of amplified nucleic acid is quantitated; and the amount of amplified nucleic acid is correlated with the number of tumor cells in the body fluid. As discussed in the "Remarks" section above, there is basis for such an amendment in the specification, which describes that the number of tumor cells circulating in a body fluid can be determined by quantitating the tumor cell mRNA that codes for the catalytic subunit of telomerase. As further discussed in the "Remarks" section above, the correlation between the amount of amplified mRNA encoding a gene and the number of cells expressing that gene was routine and well-

known to those of skill in the art at the earliest priority date of the instant application. The specification also exemplifies such correlation *e.g.*, in Figures 5 and 8, as discussed above.

In addition, as also discussed in the interview of August 25, 2003, the claims as pending herein either specify the density of the cell separation medium as being in the range of 1.060-1.067 g/ml (Claim 1 and dependents as amended herein), or recite a step of separating tumor cells from telomerase positive non-tumor cells (Claim 70, and Claim 71 and dependents). As the specification describes, *e.g.*, at page 17, line 22 to page 18, line 14, separation of the tumor cells from telomerase positive non-tumor cells as provided by the methods herein allows any subsequently detected telomerase expression from the fraction of concentrated cells to be unambiguously assigned to the tumor cells in that fraction. The cited passage of the specification further describes that such separation can be achieved by adjusting the density of the cell separation medium to a range of between 1.060-1.067 g/ml.

As discussed below, neither Cech *et al.* nor Van Vlasselaer *et al.*, singly or in combination, teaches or suggests any method that includes a step of determining the number of tumor cells in a body fluid (*i.e.*, quantification of tumor cells). Further, neither of these references, singly or in combination, teaches or suggests separation of tumor cells in a body fluid from telomerase positive non-tumor cells so that a correlation between the amplified catalytic subunit of telomerase and the number of tumor cells in the body fluid can be made.

The combination of teachings of the cited references does not result in the instantly claimed methods.

The combination of teachings of the cited references does not result in the instantly claimed methods. Neither of the cited references, singly or in combination, teaches or suggests any method for quantifying circulating tumor cells in a body fluid (all pending claims). Further, neither reference teaches or suggests the concentration of tumor cells regardless of tumor cell type using a

cell separation medium, nor the separation of tumor cells from telomerase-positive non-tumor cells in body fluids such as blood (claims 71, 69, 70). Therefore, the combination of the cited references, each of which is missing critical elements of the instant claims, cannot result in the claimed methods.

Cech *et al.* teaches quantification of the gene encoding the catalytic subunit of telomerase as indicative of cancer when the level of expression in a biological sample is elevated relative to normal levels in that sample. Cech *et al.* does not teach or suggest any step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid.

Further, Cech *et al.* does not teach or suggest any step of separating tumor cells from telomerase positive non-tumor cells. The Examiner alleges that Cech *et al.* specifically teaches that "cells or tissues may be fractionated before analysis." There is, however, absolutely no teaching or suggestion in Cech *et al.* of fractionating cells contained in a biological sample (such as a body fluid) such that in the fraction containing tumor cells, the only cells expressing telomerase are the tumor cells and not any non-tumor cells that may be present in that same fraction. As the specification describes, it is the instantly claimed methods that allow for the concentration of tumor cells from a body fluid such as blood such that even if any non-tumor cells are present along with the tumor cells, none of the non-tumor cells express telomerase. As the specification also describes, for example, at page 17, lines 31-38, the instant methods separate telomerase positive hematopoietic (non-tumor) cells from telomerase negative hematopoietic cells so that only the telomerase negative hematopoietic cells are present in the fraction containing tumor cells. This allows the unambiguous assignment of telomerase expression to the tumor cells in the fraction, and their subsequent accurate quantitation. Cech *et al.* does not teach or suggest any such step of separating tumor cells from telomerase positive non-tumor cells, much less that a particular range of cell separation medium density (*i.e.*, 1.060-1.067 g/ml) can achieve such separation.

Van Vlasselaer, directed to a method for enriching breast tumor cells from body fluids, does not cure these deficiencies. Van Vlasselaer provides a method for the concentration of breast cancer cells from a body fluid, in which the density of the gradient is adjusted according to breast cancer cell type. Van Vlasselaer does not teach or suggest quantification of any tumor cells. Further, there is no teaching or suggestion in Van Vlasselaer of concentrating tumor cells in a fraction such that the only cells expressing telomerase in that fraction are the tumor cells. Even if, as the Examiner alleges, Van Vlasselaer teaches a general method of adjusting the density of the cell separation medium according to cell type, there is no teaching or suggestion in Van Vlasselaer of fractionating a body fluid to obtain a fraction that **may contain more than one cell type** but where the only cell type expressing telomerase is the tumor cell. Finally, like Cech *et al.*, Van Vlasselaer also does not teach or suggest a particular cell separation medium density range (1.060-1.067 g/ml) that would allow for the separation of tumor cells in a body fluid from telomerase positive non-tumor cells.

Neither of the references nor their combination teaches or suggests that the catalytic subunit of telomerase can be specifically amplified and quantified from tumor cells that are concentrated in a fraction such that the only cells expressing telomerase in that fraction are the tumor cells, and that such quantitation can be used as a diagnostic assay of metastasis by quantifying the number of circulating tumor cells in a body fluid. Neither reference teaches or suggests assays of any sort for the quantification of tumor cells in general.

The cited references, singly or in combination, fail to teach or suggest several missing elements of the claims, including a step of correlating the amount of amount of amplified mRNA coding for the catalytic subunit of telomerase with the number of tumor cells expressing telomerase, and a step of separating tumor cells from telomerase positive (but not necessarily telomerase negative) non-tumor cells so that telomerase expression can be unambiguously

correlated with the number of tumor cells. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

B. REJECTION OF CLAIM 3 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF GWYNN *ET AL.*

Claim 3 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Gwynn *et al.* (U.S. Patent No. 6,025,156). It is alleged that it would have been *prima facie* obvious to one of skill in the art to have modified the method of generating cDNA for the catalytic subunit of telomerase allegedly taught by Cech *et al.* with using DNAse for the removal of DNA from RNA samples as allegedly taught by Gwynn *et al.*, to arrive at the subject matter of Claim 3. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

As discussed above, the claims have been amended to recite a positive step of "correlating the amount of amplified nucleic acid with the number of tumor cells." Further, Claim 3 is indirectly dependent on Claim 1, which specifies the density of the cell separation medium as being in the range of 1.060-1.067 g/ml, such density effecting the separation of tumor cells from telomerase positive non-tumor cells.

As discussed above, Cech *et al.* does not teach or suggest any method for the quantification of circulating tumor cells in a body fluid, much less a step of correlating expression of the catalytic subunit of telomerase from the tumor cells with the number of tumor cells. Further, as also discussed above, Cech et al. does not teach or suggest separating the tumor cells from telomerase positive non-tumor cells, nor a cell separation medium density to effect such separation. Gwynn *et al.*, directed to Topoisomerase III, does not cure these deficiencies. Even if, as the Examiner alleges, Gwynn *et al.* teaches treatment of mRNA samples with DNAse prior to reverse transcription to obtain cDNA, Gwynn *et al.* does not provide any teaching for the detection and quantification

of tumor cells, much less any of the steps of the methods as instantly claimed. Therefore, a combination of the cited references, both of which lack the elements of correlating the amount of amplified nucleic acid with the number of cells and separating tumor cells from telomerase positive non-tumor cells, cannot cure these deficiencies with respect to the instant claims. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

C. REJECTION OF CLAIM 29 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF SELBY

Claim 29 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Selby (Great Britain Patent No. GB 2 260 811) It is alleged that it would have been *prima facie* obvious to one of skill in the art to combine the method of Cech *et al.* with Selby, which allegedly teaches diagnosis or monitoring of cancer by the detection of mRNA in a sample such as blood where the cancer cells are not normally present, to arrive at the subject matter of Claim 29. It is also alleged that Selby teaches cooling after centrifugation, which is an element of Claim 29, was routinely practiced in the art. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Claim 29 specifies that in the method of Claim 1, after centrifugation and before collecting the tumor cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers. As discussed above, Claim 1 as amended herein specifies a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in the body fluid. Claim 1 as amended herein further specifies that the density of the cell separation medium is adjusted to a range of 1.060-1.067 g/ml.

As further discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are

concentrated from a body fluid by treatment with any cell separation medium, much less a cell separation medium of specified density for separating tumor cells from telomerase positive non-tumor cells as set forth in Claim 29. Cech *et al.* also does not teach or suggest any step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid. Selby, directed to diagnosis or monitoring of cancer using coamplification to detect and quantify tissue-specific genes, does not cure these deficiencies.

Even if, as the Examiner alleges, Selby teaches cooling samples after centrifugation, none of its teachings cure the deficiencies in the teachings of the Cech *et al.* so that their combination fails to provide a method for the quantification of tumor cells in a body fluid as instantly claimed. The combination of the cited references, both of which lack the elements of correlating the amount of amplified nucleic acid with the number of cells and separating tumor cells from telomerase positive non-tumor cells, cannot cure these deficiencies with respect to the instant claims. Therefore, the Examiner has failed to set forth a case of *prima facie* obviousness.

D. REJECTION OF CLAIMS 5 AND 6 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF GELMINI *ET AL.*

Claims 5 and 6 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Gelmini *et al.* (*Clin. Chem.*, 43(5): 752-758 (1997)). It is alleged that it would have been *prima facie* obvious to one of skill in the art to combine the method of Cech *et al.* with the amplification method of Gelmini *et al.*, which allegedly teaches real time quantitative PCR. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Dependent Claim 5 specifies that in the method of Claim 1 for the quantification of tumor cells in a body fluid, the amplification products of the

catalytic subunit of telomerase are labeled during amplification and the amplification kinectics are measured continuously, including during the amplification process. Claim 6 further specifies that a probe that is specific for the amplification products and that emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification. As discussed above, Claim 1 as amended herein specifies a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in the body fluid. Claim 1 as amended herein further specifies that the density of the cell separation medium is adjusted to a range of 1.060-1.067 g/ml.

As further discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment with any cell separation medium, much less a cell separation medium of specified density for separating tumor cells from telomerase positive non-tumor cells as set forth in Claims 5 and 6. Cech *et al.* also does not teach or suggest any step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid. Gelmini *et al.*, directed to quantitative PCR of the c-*erb*B-2 oncogene in breast cancer cells, does not cure these deficiencies.

Even if, as the Examiner alleges, Gelmini *et al.* teaches quantitative PCR using labelled amplification products, Gelmini *et al.* does not provide any teaching for the detection and quantification of tumor cells, much less any of the steps of the methods of Claims 5 and 6. Gelmini *et al.* also does not teach or suggest treatment with a cell separation medium, much less a cell separation medium of specified density of the range of 1.060-1.067 g/ml, and Gelmini *et al.* certainly does not teach or suggest any method, including one that employs a cell separation medium of density 1.060-1.067 g/ml, that recites a step of separating tumor cells from telomerase positive non-tumor cells. Therefore, a combination of the cited references, both of which lack the elements of (i) concentrating tumor cells from a body fluid by treatment and/or centrifugation

with a cell separation medium of density of 1.060-1.067 g/ml; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from the tumor cells in a body fluid and correlating the quantity of amplified mRNA with the number of tumor cells, cannot cure these deficiencies with respect to Claims 5 and 6. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

E. REJECTION OF CLAIM 13 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.*

Claim 13 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* It is alleged that it would have been *prima facie* obvious to one of skill in the art to select primers to amplify all or part of the catalytic subunit of telomerase gene using the parameters according to "routine methods" allegedly taught by Cech *et al.* Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Claim 13 specifies that in the method of Claim 1 for the quantification of tumor cells in a body fluid, the amplification primers whose sequences are set forth in SEQ ID. NOS. 1 and 2 are used for the amplification of the catalytic subunit of telomerase mRNA. As discussed above, Claim 1 as amended herein specifies a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in the body fluid. Claim 1 as amended herein further specifies that the density of the cell separation medium is adjusted to a range of 1.060-1.067 g/ml.

As further discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment with any cell separation medium, much less a cell separation medium of specified density for separating tumor cells from telomerase positive non-tumor cells as set forth in Claim 13. Cech *et al.* also does not teach or suggest any step of correlating the amount of

amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid.

It is alleged that the primers whose sequences are set forth in SEQ ID. NOS. 1 and 2 and that are elements of Claim 13 were merely selected by the "routine methods" provided by Cech *et al.* for the amplification of all or part of the catalytic subunit of telomerase. As discussed above, however, Cech *et al.* does not teach or suggest principal elements of Claim 13, such as a method for quantifying circulating tumor cells in a body fluid that includes a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells; or adjusting the cell separation medium to a particular density range to effect separation of tumor cells from telomerase positive non-tumor cells. Therefore, even if, as the Examiner alleges, Cech *et al.* provides primers for the quantitative amplification of the catalytic subunit of telomerase and parameters for their selection, Cech *et al.* does not provide any of the principal elements of Claim 13 as set forth above. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

F. REJECTION OF CLAIMS 12 AND 57-59 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF MELVIN *ET AL.*

Claims 12 and 57-59 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Melvin *et al.* (WO 97/12246). It is alleged that it would have been *prima facie* obvious to one of skill in the art to have modified the method of Cech *et al.* to include controls such as β-actin as a positive control and sterile water as a negative control as allegedly taught by Melvin *et al.*, to arrive at the claimed subject matter. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Claim 12 is directed to the method of Claim 1 in which water is employed as a negative control. Claim 57 is directed to the method of Claim 11, which specifies that the sample used in the method of Claim 1 is peripheral blood.

Claim 57 specifies that as a positive control, a nucleic acid that occurs in peripheral blood is specifically amplified and detected. Claim 58 further specifies positive controls such as β-globin, glyceraldehyde phosphate dehydrogenase, β-actin or a T-cell receptor. Claim 59 is directed to the method of Claim 3 where no reverse transcription reaction is carried out before amplification and/or water is used in the amplification as a negative control. All of the claims either directly or indirectly depend from Claim 1.

As discussed above, Claim 1 as amended herein specifies a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in the body fluid. Claim 1 as amended herein further specifies that the density of the cell separation medium is adjusted to a range of 1.060-1.067 g/ml.

As further discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment with any cell separation medium, much less a cell separation medium of specified density for separating tumor cells from telomerase positive non-tumor cells. Cech *et al.* also does not teach or suggest any step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid.

As discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment and/or centrifugation with a cell separation medium. Cech *et al.* also does not teach or suggest any method that includes a step of separation of tumor cells from telomerase positive non-tumor cells, much less a particular density of a cell separation medium that will effect such separation. Melvin *et al.*, directed to the detection of CYP1B1 in cancer cells, does not cure these deficiencies. Even if, as the Examiner alleges, Melvin *et al.* teaches the use of β-actin and sterile water as positive and negative controls respectively in RT-PCR experiments, Melvin *et al.* does not provide any teaching for the detection and quantification of tumor cells, much less any of the steps of the

methods of Claims 12 and 57-59. Therefore, a combination of the cited references, both of which lack the elements of (i) concentrating tumor cells from a body fluid by treatment and/or centrifugation with a cell separation medium of density 1.060-1.067 g/ml; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from the tumor cells in a body fluid and correlating the amount of amplified mRNA with the number of tumor cells, cannot cure these deficiencies with respect to Claims 12 and 57-59. Neither of the references nor their combination teaches or suggests that the catalytic subunit of telomerase may be specifically amplified and quantified in tumor (as opposed to non-tumor) cells, and that such quantitation may be used to detect and quantify tumor cells in body fluids as a diagnostic assay.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

G. REJECTION OF CLAIMS 30-33, 65 AND 66 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND OKA *ET AL.*

Claims 30-33, 65 and 66 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and further in view of Oka *et al.* (U.S. Patent No. 5,298,165). It is alleged that it would have been *prima facie* obvious to one of skill in the art to have modified the method of Cech *et al.* for the quantitation of tumor cells in view of the enrichment of tumor cells allegedly taught by Van Vlasselaer, with different membranes, filters or porous barriers allegedly taught by Oka *et al.*, to arrive at the subject matter of the rejected claims. It is further alleged that the pore size and thickness of filters are "routinely optimizable" based upon the desired parameters, since Oka *et al.* allegedly teaches how densities may be determined. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Claim 30 is directed to the method of Claim 1 in which the centrifugation vessel is divided into two compartments by a porous barrier, filter or sieve, and the body fluid is introduced into the upper compartment. Claims 31, 32, 65 and 66 specify the pore size of the porous barrier, filter or sieve, and Claim 33 specifies that at least one of the porous barrier, filter or sieve is fabricated from or coated with a hydrophobic material. All of the rejected claims are either directly or indirectly dependent on Claim 1.

As discussed above, Claim 1 as amended herein specifies a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in the body fluid. Claim 1 as amended herein further specifies that the density of the cell separation medium is adjusted to a range of 1.060-1.067 g/ml.

As discussed above, neither Cech *et al.* nor Van Vlasselaer, singly or in combination, teaches or suggests a method for the quantification of tumor cells in a body fluid where the tumor cells are concentrated from the body fluid by treatment with a cell separation medium of density 1.060-1.067 g/ml, nor of correlating the amount of amplified mRNA encoding the catalytic subunit of telomerase with the number of tumor cells in a body fluid. Oka *et al.*, directed to removal of leukocytes from leukocyte-containing blood products to reduce the side effects of blood transfusions, fails to cure these deficiencies. Oka *et al.* does not teach or suggest any assay for the detection of tumor cells in a body fluid, nor does Oka *et al.* teach or suggest quantification of tumor cells, much less separating telomerase-expressing tumor cells from telomerase-expressing non-tumor cells. While Oka *et al.* may provide for the use of filters in its methods, Oka *et al.* does not teach or suggest concentration of tumor cells in a body, much less by treatment and/or centrifugation with a cell separation medium of a particular density. Further, Oka *et al.* does not teach or suggest any method of correlating the number of cells with the amount of a gene expressed by those cells. Hence, Oka *et al.* does not cure the principal deficiencies in the teachings of Cech *et al.* and Van Vlasselaer.

The combinations of teachings fail to suggest several elements of the claimed methods, including but not limited to, concentration of tumor cells from a body fluid by treatment and/or centrifugation with a cell separation medium of density in a particular range that separates tumor cells from telomerase positive non-tumor cells, and correlating the emount of catalytic subunit of telomerase specifically amplified from tumor cells with the number of tumor cells in a body fluid. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Judicial Notice

Responsive to Applicant's arguments in the Amendment filed May 6, 2003, responsive to the previous Office Action, the Examiner alleges that Applicant's response may have "mistaken routine optimization for judicial notice." Specifically, the Examiner alleges the following: (1) with regard to Applicant's arguments that the use of Percoll and Ficoll as cell separation media; providing a substance that prevents platelets from sticking to the tumor cells and facilitates removal of the platelets; and adjusting the density of the cell separation medium according to cell type as applied to the instant methods is inappropriate judicial notice, the Examiner alleges that specific sections of Van Vlasselaer were cited for the proposition that the aforementioned steps are "routine." ; (2) with regard to Applicant's arguments that cooling following centrifugation as a "routine" practice is inappropriate judicial notice, it is alleged that Selby was cited for the specific teaching that such a step is routine ; (3) with regard to Applicant's arguments that the design of primers as being "routine" is inappropriate judicial notice, the Examiner alleges that Cech *et al.* specifically teaches how to design primers ; (4) with regard to Applicant's arguments that the use of particular pore sizes and thicknesses in the instantly claimed methods is inappropriate judicial notice, it is alleged that Oka *et al.* provides numerous different filters with different thicknesses, and also teaches the average pore sizes of filters (col. 10, lines 40-45) and, therefore, the use of particular pore sizes and thicknesses is a matter of "routine experimentation."

As discussed below and in the previous response, it is maintained that the Examiner cannot take official notice of facts outside the record that are not capable of instant and unquestionable demonstration.

The Examiner is reminded that MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . .. (emphasis added).

MPEP 2144.03 goes on to provide, as examples of facts of which proper official notice can be taken, the "common practice to postheat a weld after the welding operation is completed, or "to adjust the intensity of a flame in accordance with the heat requirements" (*In re Ahlert*). These facts are in the nature of universal truths that are uniformly applicable and are guaranteed to work, irrespective of variations, in a given art.

To the contrary, the elements that the Examiner urges any one of ordinary skill in the art would have done in the context of the subject matter of this application are not "capable of instant and unquestionable demonstration" as being "well-known" in the art. The "routine art" cited by the Examiner involves experimentation with various sets of conditions to optimize the steps of the claimed methods. Further, contrary to the Examiner's assertions, this optimization is not routine. While the steps recited in Van Vlasselaer are applicable to the specific method in Van Vlasselaer of concentrating a single cell type, namely, breast cancer cells from a body fluid, there is no teaching in Van Vlasselaer that these steps are universally applicable to any method involving concentration steps, particularly a method as instantly claimed where the tumor cells are fractionated such that even if other cells are present along with the tumor cell fraction, only the tumor cells express telomerase.

The "routine art" cited by the Examiner involves experimentation with various sets of conditions to optimize the steps of the claimed methods. This experimentation is non-routine over Van Vlasselaer, given that unlike Van Vlasselaer, where a single cell type is separated, the instant separation of a

tumor cell enriched fraction may contain more than one type of cell as long as only the tumor cells expresss telomerase. For example, the selection of density of the cell separation medium to separate tumor cells from telomerase positive non-tumor cells is neither taught or suggested by the "routine" concept of choosing a density according to the characteristics of a particular type of cell. It is not enough that adjusting medium density according to a cell type is known; the optimal densities for concentration of tumor cells from a body fluid such that only the tumor cells express telomerase regardless of cell type are only discovered through experimentation. Thus, the density of cell separation medium that produces a workable step in the claimed methods is neither "routine" nor "well-known". Further, the use of a substance that prevents platelets from sticking to tumor cells before concentrating the tumor cells does not automatically render the ability to detect a particular target gene, such as the catalytic subunit of human telomerase as claimed in the instant application, nor the quantification of tumor cells, obvious. The selection of a purification technique for biological samples is also neither "routine" nor "Well-known"; such selection is based on the nature of the sample, desired results and available equipment.

Similarly, the practice of cooling the samples after centrifugation has been shown to be applicable to the instantly claimed method for fractionating tumor cells such that, regardless of other cells present in the tumor cell fraction, only the tumor cells express telomerase, only through Applicant's own efforts. Selby does not teach or suggest how a fractionation where only one type of cell contained in the fraction (the tumor cell) expresses telomerase may be effected by centrifugation. Further, the success of the primers chosen for amplification of a gene in the context of a method, such as the instant method involving concentration and quantification of tumor cells in a body fluid wherein the concentrated fraction may contain cells other than the tumor cells, depends largely on the particular primers selected, which is subject to experimental trial and error that is particular to the instantly claimed methods.

Cech *et al.* provides no teaching or suggestion as to selection of particular primers for an assay for the quantification of tumor cells in a body fluid. It is not enough that the sequence of the target nucleic acid to be amplified or of suggested lists of primer sequences and their preferred lengths be known; the optimal primer sites for specific amplification of the catalytic subunit of telomerase from the tumor cells separated as instantly claimed are only discovered through experimentation with various primer sequences. Moreover, as discussed above, Cech *et al.* does not even provide the primer sequences that are elements of Claim 13 as part of its list of suggested sequences from which they could be "selected". Thus, the construction of primers that produce a workable step in the claimed subject matter is neither "routine" nor "well-known. The above "routine art" has been shown to be applicable to the instantly claimed method for the quantification of tumor cells in a body fluid only through Applicant's own efforts.

Finally, there is no teaching or suggestion in Oka *et al.* that the selection of an optimum purification technique for enriching or depleting tumor cells from a body fluid so that only the tumor cells in the enriched fraction express telomerase (*i.e.*, no telomerase-expressing non-tumor cells are present in the enriched fraction) would be "routine;" such selection is based on the nature of the sample, desired results and available equipment.

Therefore, a reference or references supporting assertions that the steps recited in Van Vlasselaer, Cech *et al.*, Oka *et al.* and Selby are "routine" as applied to a method in which cells are separated, not by particular cell type, but such that only the tumor cells in the separated fraction express telomerase, should be provided. But, even if such reference(s) were provided, it would not establish obviousness because none of the references, "routine art" nor any combinations thereof teach or suggest those elements that are present in all the pending claims, namely, the quantification of tumor cells by correlating the amount of amplified catalytic sybunit of telomerase with the number of tumor cells in a body fluid or separating the tumor cells from telomerase positive non-

tumor cells by, *e.g.*, adjusting the density of the cell separation medium to a particular range (1.060-1.067 g/ml).

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the remarks herein, reconsideration of the requirement for restriction and examination of all claims on the merits are respectfully requested.

Respectfully submitted,
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